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Rapid Manual Immunoturbidimetric and Immunonephelometric Assays of Prealbumin, Albumin, IgG, IgA and IgM in Cerebrospinal Fluid

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Summary: Manual turbidimetric kinetic assays (10 to 20 samples/h) for the determination of prealbumin, albumin, IgG and IgA in CSF, and a turbidimetric end point assay for IgM are described for a mechanized microliter system (Eppendorf); it uses microcuvettes (total volume 300 µl) and polyethylene glycol to increase sensitivity (detection limit 1 to 3 mg/l) and reaction velocity (⩽ 12 min and 25 min, respectively). The results correlated satisfactorily with those obtained with laser end point nephelometry (1–2 h, total volume with Hyland laser 750 µl, with Behring laser 225 µl), e.g. correlation coefficient of > 0.9 for comparison of CSF samples, recovery rates ~90 to 110%, day to day precision < 10%. No interference with other proteins was observed (e.g. hemoglobin). The turbidimetric assays were generally less expensive with respect to reagents (by factor 2 to 20), and they required smaller sample volumes (by factor 2) and less technician time (by factor 2 to 4, especially for number of samples ⩽ 10) than both nephelometric end point assays, because they do not need daily established calibration curves. The turbidimetric kinetic assay in series to 6 samples can be compared to the Beckman immunochemistry analyser which requires still less time, but is less sensitive and more cost-intensive.

Schnelle manuell-immunoturbidimetrische und immunonephelometrische Tests für Präalbumin, Albumin, IgG, IgA und IgM im Liquor cerebrospinalis

Zusammenfassung: Manuell turbidimetrisch-kinetische Tests (10 bis 20 Proben/h) für die Bestimmung von Präalbumin, Albumin, IgG und IgA im Liquor und ein turbidimetrischer Endpunkt-Test für IgM werden für ein mechanisiertes Mikrolitersystem (Eppendorf) beschrieben, welches Mikroküvetten (Gesamtvolumen 300 µl) benötigt und Polyethylenglycol, um die Empfindlichkeit (Nachweisgrenze 1–3 mg/l) und Reaktionsgeschwindigkeit (⩽ 12 min bzw. 25 min) zu erhöhen. Die Ergebnisse korrelierten zufriedenstellend mit mittels Laser-Endpunkt-Nephelometrie erhaltenen (1–2h, Gesamtvolumen für Hyland Laser 750 µl, für Behring Laser 225 µl): Korrelationskoeffizient > 0.9 beim Vergleich von Liquorproben, Wiederauffindung ~90–110%, Tag-zu-Tag-Präzision < 10%. Keine Störungen durch andere Proteine wurden beobachtet (z.B. Haemoglobin). Trübungstests benötigen im allgemeinen 2- bis 20-mal weniger Reagenzienkosten, zweimal weniger Probenvolumen und 2- bis 4-mal weniger Zeit zur Durchführung (besonders bei kleiner Probenanzahl ⩽ 10) als beide nephelometrischen Endpunkt-Tests, weil sie keine täglich erstellte Standardkurve brauchen. Der turbidimetrisch-kinetische Test in Serien zu 6 Proben kann mit dem Beckman Immunochemistry Analyser verglichen werden, welcher noch weniger Zeit erfordert, aber weniger empfindlich ist und kostenintensiver arbeitet.

Introduction

The determination of proteins in human serum by using their specific antisera has been described by applying nephelometric (1–4) or turbidimetric procedures (5–7) which detect the antigen-antibody complex formed in the presence of antibody excess. However, proteins in CSF exhibit considerably lower concentration levels than in serum (by a factor of 100 to 5000) and generally the procedures described are less sensitive, so that they have to be adapted to the conditions in CSF. One possibility is to increase the volume of the native sample which should be used unconcentrated on account of possible loss and denaturing of protein during concentration processes. Another possibility is to add polyethylene glycol to the reaction mixture in order to increase sensitivity of antigen-antibody reaction and to linearize precipitin curves (8). Moreover, a nephelometer

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with highly sensitive photomultipler of variable amplitude can be used (e.g. Hyland laser nephelometer) to increase sensitivity.

In this study manual turbidimetric and nephelometric techniques for the detection of prealbumin, albumin, IgG, IgA and IgM were adapted to CSF. Preliminary results of this study have been published recently (9). For comparison, measurements with the Hyland laser nephelometer were used as reference values. Operating processes already exist which take into consideration different blanks for the determination of albumin and immunoglobulins in human CSF (10). The CSF sample is diluted by a large volume of antibody solution containing only small amounts of polyethylene glycol (2 g/l), thus preventing unspecific effects.

Materials and Methods

Materials

Monospecific antisera and LN antisera from rabbits against human prealbumin, albumin, IgG, IgA and IgM as well as standard sera and control sera and LN standard sera were obtained from Behringwerke Marburg/L. M-Partigen immunodiffusion plates for the determination of prealbumin were supplied by Behringwerke Marburg/L.. Monospecific antisera from goats against albumin, IgG, IgA and IgM as well as standards were a kind gift from Hyland-Travenol München. Monospecific antisera from sheep against IgG, IgA and IgM (Tina quant) as well as standards were a kind gift from Boehringer Mannheim G.m.b.H.. Monospecific antisera from goats against IgG, IgA and IgM as well as different standards were a kind gift from Beckman Instruments, München.

All standards were diluted with sterile 0.15 mol/l NaCl (saline) and stored at 4°C (for dilution scheme see figs. 1–5). Polyethylene glycol 6000 was obtained from Serva, Heidelberg, and dissolved in buffers A: 0.05 mol/l NaH_2PO_4, 0.15 mol/l NaCl, 1 µmol/l merthiolate, 50 g/l polyethylene glycol; B: 0.09 mol/l NaH_2PO_4, 0.27 mol/l NaCl, 1.8 µmol/l merthiolate, 90 g/l polyethylene glycol; C: 0.18 mol/l NaH_2PO_4, 0.54 mol/l NaCl, 3.6 µmol/l merthiolate 180 g/l polyethylene glycol. All reagents were of best analytical grade and dissolved in fresh quartzbidistilled water. Care was taken, that all solutions and antisera used were free of dust particles and brought to room temperature. For the Hyland laser nephelometry all solutions were filtered through Membrane filters (SM 11605, 0.45 µm pore size from Sartorius Werke, Göttingen).

Specimen collection

Mainly samples of lumbar liquores were used which were freed of cells by a membrane filtration technique (SM 11605, pore size 0.6 µm, Sartorius Werke, Göttingen, cf. l.c. (11)) and stored at 4°C in sterile polystyrene tubes with screw cups.

Equipment

In addition to the Eppendorf microliter system, the following apparatus was used:

Hyland laser nephelometer PDQ™ (632.8 nm, angle 31°) using glass tubes (Hyland Travenol, München);

Behring laser nephelometer (632.8 nm, forward scattering) with Modul I plus Modul II coupled to Hewlett Packard Computer 9815 A (Hewlett Packard, Frankfurt/M.). LN universal cuvettes were used (Behringwerke, Marburg/L.);

Beckman immunochemistry analyser (400–500 nm, angle 70°) using 600 µl reaction mixture, start with 42 µl sample (Beckman Instruments, München).

All nephelometric assays were performed at room temperature.

Eppendorf enzyme analyser 5086 with thermo-regulated glass microcuvettes (25°C, d = 1 cm, total volume 300 µl) (Eppendorf Gerätebau, Hamburg). Measurements in series of six cuvettes were performed at 365 nm. Recording time for each cuvette was 12 s per one passage.

Methods

Procedures for the detection of prealbumin, albumin, IgG, IgA, and IgM by four techniques are listed up in tables 1–4.

Calculation of results

For each series with every procedure a calibration curve was established consisting of 7 standard values in duplicate for the whole assay range. The values obtained with the Hyland laser nephelometer were corrected for the three blanks according to the instructions of the manufacturer. For Behring laser nephelometer each sample value was subtracted from its individual blank calculated in mg/l. For both nephelometric procedures 10 to 20% of CSF samples (n = 429) had individual blanks > 1 mg/l protein, 20 to 30% of them had blanks > 0.5 mg/l. The blanks depended upon sample volume. Samples were discarded when their sample blank exceeded 30% of the sample value. For turbidimetric end point analysis each sample value was corrected for the serum blank. Turbidimetric kinetic analysis yielded blanks ± 0, but they

Tab. 1. Experimental set up for assays by Hyland laser nephelometer (total volume 0.725–0.750 ml).

Antiserum and solutions	Sample (ml)	Sample blank (ml)	Antibody blank (ml)	NaCl blank (ml)
Reaction mixture for				
Albumin	0.700	–	0.700	–
IgG	0.700	–	0.700	–
IgA	0.650	–	0.650	–
IgM	0.600	–	0.600	–
Cell free CSF[a]) for				
Albumin	0.050	0.050	–	–
IgG	0.025	0.025	–	–
IgA	0.100	0.100	–	–
IgM	0.150	0.150	–	–
0.15 mol/l NaCl for				
Albumin	–	0.700	0.050	0.750
IgG	–	0.700	0.025	0.725
IgA	–	0.650	0.100	0.750
IgM	–	0.600	0.150	0.750

[a]) or standards from Hyland Travenol, or control serum from Behringwerke. Reaction mixture according to Hyland Travenol, München. Reaction is started by adding antiserum and carried out further for 1 h with albumin and IgG, for 2 h for IgA and IgM at room temperature in closed tubes. At first the apparatus is adjusted with NaCl blank and antibody blank, then sample blank and sample are measured according to the instructions of Hyland Travenol by keeping 15 s interval throughout!

Tab. 2. Experimental set up for assays by the Behring laser nephelometer (total volume 0.225 ml).

Antiserum and solutions	Sample (ml)	Sample blank (ml)	Reagent blank (ml)
Buffer A for			
Prealbumin	0.050	0.110	0.050
Albumin	0.100	0.160	0.100
IgG	0.100	0.160	0.100
0.15 mol/l NaCl for			
Prealbumin	0.050	0.065	0.100
Albumin	–	0.015	0.050
IgG	–	0.015	0.050
IgA	–	0.075	0.150
IgM	–	0.075	0.150
Cell free CSF[a]) for			
Prealbumin	0.050	0.050	–
Albumin[b])	0.050	0.050	–
IgG	0.050	0.050	–
IgA	0.150	0.150	–
IgM	0.150	0.150	–
Antiserum diluted 1+4 for			
Prealbumin	0.075[c])	–	0.075[c])
Albumin	0.075[c])	–	0.075[c])
IgG	0.075[c])	–	0.075[c])
IgA	0.075[d])	–	0.075[d])
IgM	0.075[d])	–	0.075[d])

[a]) or standard, or control serum from Behringwerke Marburg/Lahn.
[b]) the sample was prediluted 1 + 9 with saline.
[c]) 0.015 ml antiserum plus 0.060 ml buffer A.
[d]) 0.015 ml antiserum plus 0.060 ml saline.
For c and d incubation at room temperature for 0.5–1 h, followed by centrifugation at 15 000 g immediately before use. Reaction is started by adding antiserum and carried out further for 0.5–2 h with prealbumin, for 1 h with albumin and IgG, for 2 h with IgA and IgM at room temperature. Starting and measuring are done within 15 s interval. LN-antiserum and LN-standards for albumin, IgG, IgA and IgM came from Behringwerke Marburg/L. as well as antiserum for prealbumin.

exhibited a standard deviation > 0 (see determination of detection limit). From these corrected standard values a polynominal regression curve was calculated according to the formula $y = a \pm bx \pm cx^2 \pm dx^3 \pm ex^4 \pm fx^5$ with a Hewlett Packard computer 9815 A; y evaluation from standards was < 2%. The 7 standards were assayed in duplicate by Behring laser nephelometry, together with the serum blank. Sample values were calculated by this formula in mg/l or U/ml.

Results and Discussion

Determination of the detection limit and upper range of the turbidimetric and nephelometric techniques

For *prealbumin* measurements nephelometric and turbidimetric end point techniques had similar detection limits (between 1 and 2 mg/l, see tab. 5) and upper ranges (≥ 40 mg/l, see fig. 1); for turbidimetric kinetic analysis the detection limit is two times higher (table 5), as its standard curve exhibited a smaller slope than that of turbi-

Tab. 3. Experimental set up for turbidimetric end point assays (Total volume 0.3–0.32 ml).

Antiserum and solutions	Sample (ml)	Blank (ml)
Buffer A for		
Prealbumin	0.15	0.15
Albumin	0.28	0.28
IgG	0.20	0.20
Antiserum for		
Prealbumin	0.05	0.05
Albumin	0.02	0.02
IgG	0.05	0.05
Cell free CSF[a]) for		
Prealbumin	0.10	–
Albumin	0.02	–
IgG	0.05	–
0.15 mol/l NaCl for		
Prealbumin	–	0.10
Albumin	–	0.02
IgG	–	0.05

[a]) or standards, or control serum from Behringwerke Marburg/Lahn.
All cuvettes are filled and measured at 365 nm within 12 s interval. Start with sample or standards while the recorder is on. Record the absorption after 45 min for prealbumin, 15 min for albumin, 20 min for IgG.
Antiserum from Behringwerke Marburg/Lahn.

Tab. 4. Experimental set up for turbidimetric kinetic assays (total volume 0.3 ml).

Antiserum and solutions	Sample (ml)	Blank (ml)
Buffer A for prealbumin[a])	0.20	0.20
Buffer A for albumin[a])	0.02	0.02
Reaction mixture[b]) for IgG	0.20	0.20
+ buffer B	0.05	0.05
Reaction mixture[b]) for IgA concentrated by factor of 2	0.20	0.20
+ buffer C	0.05	0.05
Reaction mixture[b]) for IgM concentrated by factor of 4	0.15	0.15
+ buffer A	0.05	0.05
0.15 mol/l NaCl for		
Prealbumin	–	0.05
Albumin	0.21	0.26
IgG	–	0.05
IgA	–	0.05
IgM	–	0.10
Antiserum for		
Prealbumin[a])	0.05	0.05
Albumin[a])	0.02	0.02
Cell free CSF[c]) for		
Prealbumin	0.05	–
Albumin[d])	0.05	–
IgG	0.05	–
IgA	0.05	–
IgM	0.10	–

[a]) Antiserum and standards from Behringwerke Marburg/Lahn;
[b]) Tina quant from Boehringer Mannheim; reaction mixture is originally dissolved in 30 ml, for IgA in 15 ml, for IgM in 7.5 ml buffer dependent on the batch used.
[c]) or standard or control serum from Boehringer Mannheim;
[d]) the sample was prediluted 1 + 9 with saline.
All cuvettes are filled and measured at 365 nm within 12 s interval. Start with sample or standard while the recorder is on. Record the absorption exactly after 0.5 and 6 min for albumin, IgG and IgM, 0.5 and 12 min for prealbumin and IgA.

Tab. 5. Detection limit of two nephelometric and two turbidimetric techniques for the determination of five proteins in CSF. Detection limit is given as 3 standard deviations of the blank value acc. to l.c. (11); in parenthesis number of determinations per series. For detail of techniques see methods.

Technique	Prealbumin (mg/l)	Albumin (mg/l)	IgG (mg/l)	IgA (mg/l)	IgM (mg/l)
Hyland laser nephelometry	–	50 (10)	2 (10)	4 (10)	1 (10)
Behring laser nephelometry	1.3 (30)	10 (30)	3 (30)	1 (30)	1 (10)
Turbidimetric analysis					
End point assay	1.5 (22)	10 (36)	1 (15)	–	2 (10)
Kinetic assay	3.0 (12)	10 (12)	3 [a](6)	2[a] (12)	–

[a]) kinetic assay B

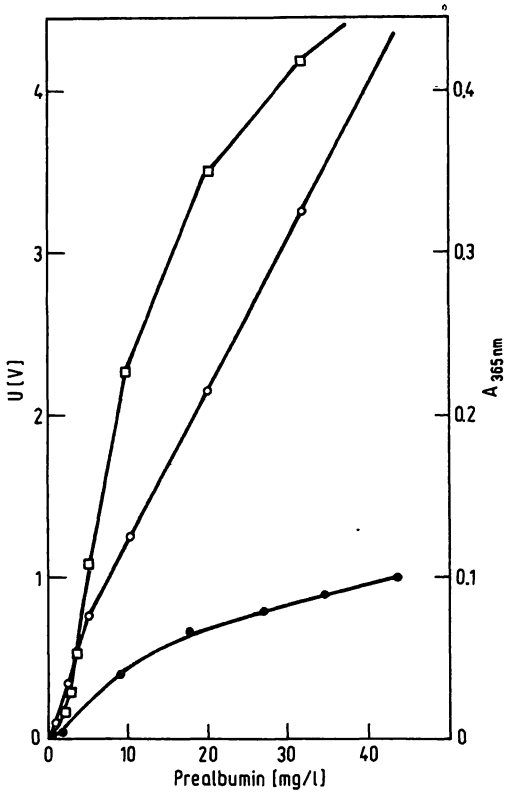

Fig. 1. Calibration curves for human prealbumin established with three immunological techniques. •——• turbidimetric kinetic assay, o——o turbidimetric end point assay, □——□ Behring laser nephelometry. For details see materials and methods and tabs. 2–4.

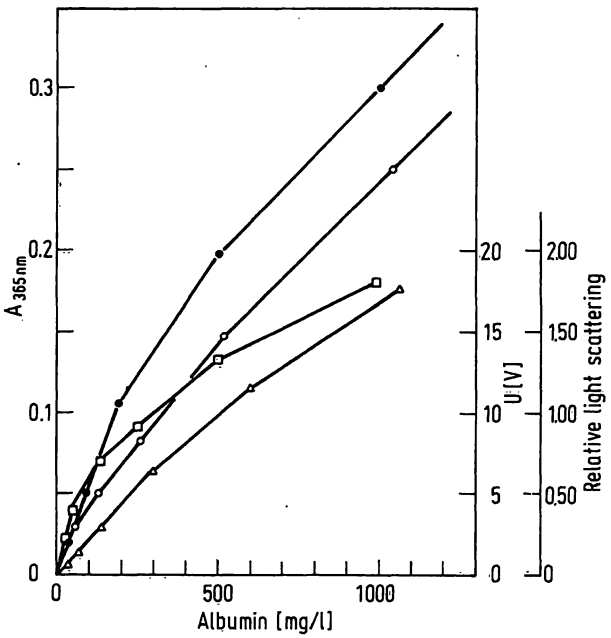

Fig. 2. Calibration curves for human albumin established with four immunological techniques. •——• turbidimetric kinetic assay, o——o turbidimetric end point assay, □——□ Behring laser nephelometry, △——△ Hyland laser nephelometry. For details see materials and methods, tabs 1–4 and fig. 1.

dimetric end point assay (fig. 1). Generally, turbidimetric kinetic assays showed mean blank values of zero, having a standard deviation from which the detection limit is taken as 3 standard deviations of the blank value (cf. l.c. (12)). Nevertheless, these kinetic assays do not need blanks.

Techniques established with the Behring laser nephelometer as well as with the Eppendorf enzyme analyser 5086 (end point and kinetic assays) for *albumin* exhibited the same detection limits of 10 mg/l (table 5) and upper ranges of about 1000 mg/l (see fig. 2). These results indicate no differences between the two techniques with antisera and LN-antisera from Behringwerke Marburg/L. The Hyland laser nephelometer procedure set up in the same range showed a higher detection limit (by a factor of 5, see table 5) and a smaller slope (fig. 2). Turbidimetric and nephelometric standard curves cannot be directly compared on account of their different units on the y-ordinate.

All the *IgG* assays had comparable detection limits (tab. 5); however, the turbidimetric end point assay yielded the lowest value and the steepest calibration curve (fig. 3) indicating no need for LN-antisera to obtain optimum standard curves. The upper range lay higher with nephelometric assays (≥ 250 mg/l, see fig. 3) than with turbidimetric kinetic assay A (< 150 mg/l) containing low concentration of polyethylene glycol. Fig. 3

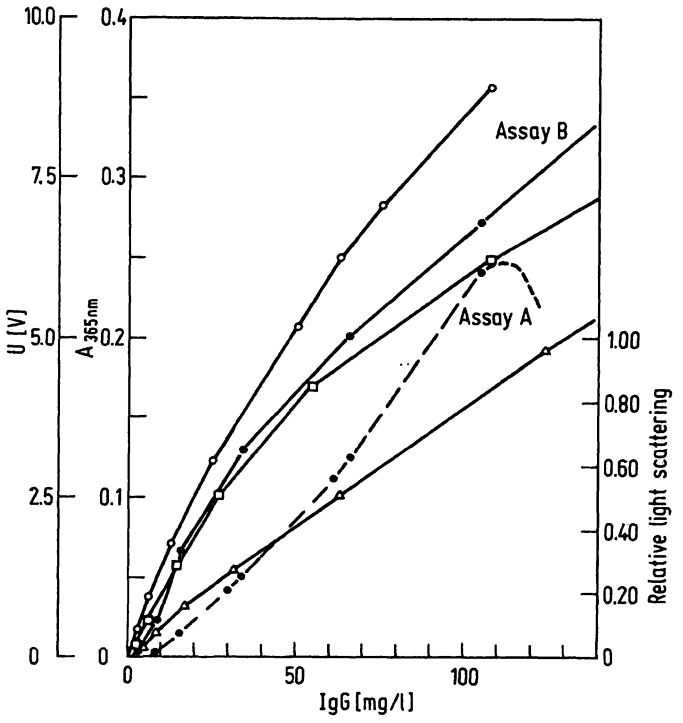

Fig. 3. Calibration curves for human IgG established with four immunological techniques. •——• turbidimetric kinetic assays A and B (Tina quant), o——o turbidimetric end point assay, □——□ Behring laser nephelometry, △——△ Hyland laser nephelometry. For details see materials and methods, tabs. 1–3 and fig. 1; assay B as described in tab. 4, assay A as assay B, but 250 µl reaction mixture without buffer B.

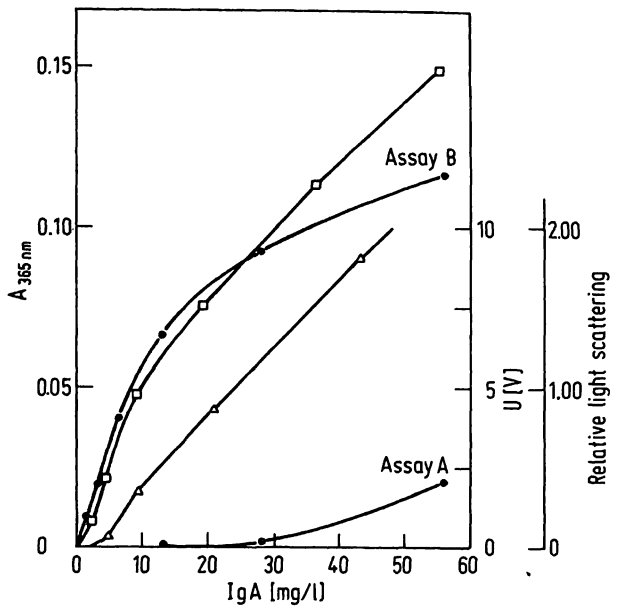

Fig. 4. Calibration curves for human IgA established with three immunological techniques. •——• turbidimetric kinetic assays A and B (Tina quant), □——□ Behring laser nephelometry, △——△ Hyland laser nephelometry. For details see materials and methods, tabs. 1, 2 and fig. 1; assay B as described in tab. 4, assay A as assay B, but 250 µl reaction mixture concentrated by a factor of 2 without buffer C.

demonstrates the effect of higher concentrations of polyethylene glycol which elevated the sensitivity and range of the calibration curve of the turbidimetric kinetic assay (compare curves from assay A with assay B, fig. 3 and cf. l.c. (8)).

From the three *IgA* assays Behring laser nephelometry and turbidimetric kinetic assay proved to have similar low detection limits (tab. 5) and upper ranges (~50 mg/l, fig. 4), whereas Hyland laser nephelometry showed a higher detection limit (by a factor of 2 to 4) and a flatter shaped calibration curve of the same range. For the turbidimetric kinetic assay high concentrations of polyethylene glycol as well as twice concentrated antiserum mixture (Tina quant) were needed to get an optimum calibration curve (see assay A and B in fig. 4).

The detection limit for three end point assays for *IgM*, two nephelometric and a turbidimetric one, was similar (see table 5) as well as the upper range of calibration curves (≥ 30 mg/l, see fig. 5). Figure 5 demonstrates that the calibration curve obtained with the original concentration of antiserum of Tina quant for an end point measurement of 25 min (assay A) was too flat and less sensitive. Only after concentrating the antiserum mixture by a factor of 4 and bringing the concentration of polyethylene glycol to 34 g/l was it possible to obtain an optimum calibration curve for 25 min end point measurements (assay B); under these conditions, however, the kinetic assay proved to be less sensitive (assay C, fig. 5). It gave a calibration curve similar to the end point measurement assay using original concentrations of antiserum mixture (see fig. 5 assay A).

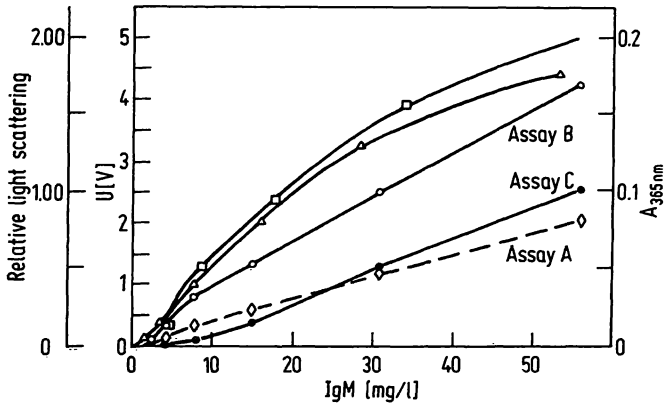

Fig. 5. Calibration curves for human IgM established with four immunological techniques. •——• turbidimetric kinetic assay (Tina quant) assay C, o——o turbidimetric end point assay B (0.5–25 min, conditions as described in tab. 4), ◇——◇ turbidimetric end point assay A (0.5–25 min, 0.20 ml original antiserum mixture (Tina quant), 0.1 ml sample volume); □——□ Behring laser nephelometry, △——△ Hyland laser nephelometry. For details see materials and methods, tabs. 1, 2, 4 and fig. 1.

Finally, all the techniques presented exhibited nonlinear standard curves over the whole range tested (figs. 1–5).

Determination of precision

Generally, turbidimetric and nephelometric techniques for the five proteins under investigation showed similar values for day-to-day precision, where lower concentrations around the detection limit of the assays exhibited greater coefficients of variation than higher concentrations of the diluted control sera (tab. 6,7). Nevertheless the values were < 10.5% in each case, also with CSF samples stored for 1–2 months at 5 °C and measured once per week. The within-run precision yielded somewhat lower values than those of day-to-day precision (not shown here).

Comparing the day-to-day precision of calibration curves, obtained by the turbidimetric kinetic and end point assays, the coefficients of variation were below the 10% limit for standards with low and high concentrations. However, this was not the case for calibration curves established by both nephelometric techniques. Here standards in the lower range exhibited coefficients of variation between 10 to 40% indicating that antigen-antibody reactions are still proceeding after 1 or 2 h of incubation. The results point to the necessity of setting up a calibration curve for each nephelometric assay, whereas this is not the case for turbidimetric assays.

The variance between desired value and actual value of each control serum lay below the 10% limit with one exception of the turbidimetric kinetic assay for IgM, where a variance of ~20% was found (tab. 6). The data indicate differences of the calibration of the different control and standard sera which should be compared to a common standard (cf l.c. (13,14)).

Tab. 6. Day-to-day precision of two turbidimetric techniques with five proteins in diluted control sera from Behringwerke Marburg/L.

Protein	$\bar{x}$ (mg/l)		CV[a] (%)	n[b]	Turbidimetric technique
Prealbumin	9.9	10.6	7.4	20	End point analysis
	39.6	38.6	5.7	20	
Albumin	47	44	10.2	20	End point analysis
	188	178	3.6	20	
IgG	6.3	6.0	8.9	20	End point analysis
	25.2	26.5	5.5	20	
IgG	5.4	5.4	5.5	18	Kinetic analysis
	53.7	58.0	6.8	18	
IgA	9.5	9.9	8.3	18	Kinetic analysis
	18.8	20.0	8.5	18	
IgM	5.8	4.6	9.2	18	Kinetic analysis
	11.5	9.5	5.7	18	

[a]) Mean value ($\bar{x}$) with coefficient of variation.
[b]) Number of days.

Tab. 7. Day-to-day precision of two nephelometric techniques with four proteins in diluted control sera from Behringwerke Marburg/L.

Protein	$\bar{x}$ (mg/l)		CV[a] (%)	n[b]	Nephelometric techniques
Prealbumin	3.6	3.9	6.3	18	Behring laser
	19.8	18.5	2.8	18	
Albumin	188	179	6.7	18	Hyland laser
	741	177	2.3	18	
IgG	26	24	10.4	18	Hyland laser
	71	77	2.4	18	
IgG	7.2	7.9	7.3	18	Behring laser
	71	78	7.0	18	
IgA	9.5	9.5	9.1	20	Behring laser

[a]) Mean value ($\bar{x}$) with coefficient of variation
[b]) Number of days

Determination of accuracy

The accuracy of immunoturbidimetric and immunonephelometric assays of five proteins in CSF was determined by the following approaches using mainly Hyland laser assays as reference (see introduction).

a) *Recovery experiments* with lumbar CSF with added pure human IgG or diluted human serum, yielded satisfactory recovery for the five proteins (tab. 8); the values obtained with turbidimetric and nephelometric techniques were comparable. Moreover, the addition of diluted human sera or other proteins (e.g. human γ-globulin, bovine serum albumin, tab. 8), gave some information on the specificity of these immunological techniques, because their presence did not interfere (two possible exceptions may be IgG and IgA recovery of turbidimetric analysis with yields between 75 and 100%, tab. 8). No interference was obtained with other proteins (cf. l.c. (8)), e.g. hemoglobin with different concentrations in turbidimetric and nephelometric assays (see tab. 8).

b) *Correlation of concentration of the five proteins measured in CSF by different techniques.* Turbidimetric kinetic and end point analysis for CSF samples were compared with Hyland or Behring laser nephelometry.

The accordance obtained between turbidimetric kinetic and nephelometric assays for *prealbumin* was more or less satisfactory (the Behring laser nephelometry yielded somewhat higher values, fig. 6), but there was also good agreement between nephelometric or turbidimetric end point analysis and radial immunoassay (y-intercept was below the detection limit of the tests, see tab. 9). Without the use of polyethylene glycol, the concentrations of which are given in the assays, the reaction of CSF ran slower than the standards yielding non-concordant values with radial immunoassay.

The concordance obtained between turbidimetric kinetic or end point analysis and nephelometric analysis for

Tab. 8. Recovery experiments with nephelometric and turbidimetric techniques for five proteins in lumbar liquor ± the presence of other proteins.

Technique	Protein added to CSF sample (mg/l)	recovery (%)	Other proteins added (mg/l)
Behring laser nephelometry	5.3 prealbumin	109 ± 5[a]	920[b]
Behring laser nephelometry	5.3 prealbumin	98 ± 8	2,222[c]
Behring laser nephelometry	5.3 prealbumin	100 ± 7	1,333[d]
Turbidimetric kinetic analysis	10.5 prealbumin	116 ± 11	240[b]
Behring laser nephelometry	112 albumin	97 ± 13	60[b]
Behring laser nephelometry	56 albumin	105 ± 7	4,889[c]
Turbidimetric kinetic analysis	45 albumin	115 ± 9	24[b]
Turbidimetric kinetic analysis	189 albumin	109 ± 8	525[f]
Hyland laser nephelometry	0.3 IgG	112 ± 16	none[e]
Hyland laser nephelometry	32.2 IgG	97 ± 6	76[f]
Behring laser nephelometry	6.8 IgG	96 ± 6	31[b]
Behring laser nephelometry	6.8 IgG	103 ± 6	2,667[d]
Behring laser nephelometry	13.1 IgG	98 ± 5	7[f]
Turbidimetric end point analysis	0.7 IgG	97 ± 4	none[e]
Turbidimetric end point analysis	108 IgG	88 ± 14	79[d]
Turbidimetric kinetic assay B	8.0 IgG	101 ± 9	35[b]
Behring laser nephelometry	4.4 IgA	96 ± 10	131[b]
Behring laser nephelometry	4.4 IgA	103 ± 6	2,667[d]
Turbidimetric kinetic assay B	3.1 IgA	89 ± 11	94[b]
Hyland laser nephelometry	8.0 IgM	98 ± 6	165[b]
Turbidimetric end point assay 25 min, 34 g/l polyethylene glycol	3.0 IgM	103 ± 12	210[b]

[a]) mean value ± S.D., n = 3–10, b) other proteins from the diluted serum added, c) human γ-globulin (Serva, Heidelberg) together with diluted serum, d) bovine serum albumin (Behringwerke, Marburg/L.) together with diluted serum, e) pure human IgG which was a kind gift from Prof. *Rother*, Inst. f. Immunologie der Universität Heidelberg, f) human hemoglobin isolated from erythrocytes after hemolysis and centrifugation at 15 000 *g*.

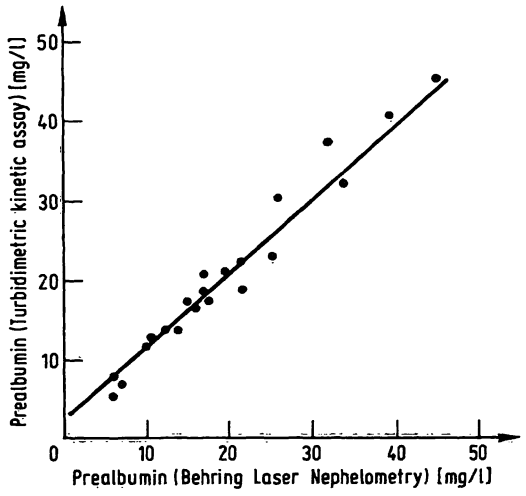

Fig. 6. Prealbumin concentrations in CSF as determined by turbidimetric kinetic assay (see tab. 4) and Behring laser nephelometry (see tab. 2) (n = 20).
Slope: 0.89, intercept: 3.39 mg/l, correlation coefficient: 0.947.

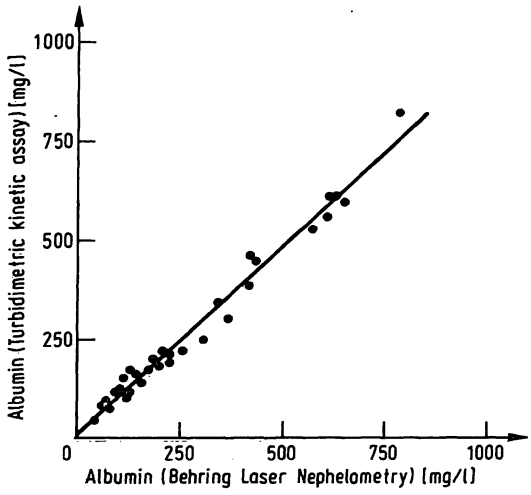

Fig. 7. Albumin concentrations in CSF as determined by turbidimetric kinetic assay (see tab. 4) and Behring laser nephelometry (see tab. 2) (n = 36).
Slope: 0.96, intercept: 12.17 mg/l, correlation coefficient: 0.978.

albumin was good (fig. 7 and tab. 9); it was satisfactory between Hyland laser nephelometry and Behring laser nephelometry (tab. 9). Here polyethylene glycol used in concentrations between 3.3 and 44 g/l increased the sensitivity and range of turbidimetric and nephelometric assays (Behring laser) without producing any interference (y-intercept was around the detection limit of the tests; see fig. 7, tab. 9).

The *IgG* concentrations determined in CSF by six different techniques correlated in different ways: a good concordance was obtained between turbidimetric end point analysis with polyethylene glycol (PEG) and Hyland laser nephelometry (tab. 9 negative y-intercept indicates a regression curve which is not linear possibly due to wrong values of blanks). It was good between turbidimetric kinetic analysis and Behring laser nephelometry

Tab. 9. Statistical comparison of immunological techniques with the regression equation y = ax ± b. For experimental details see methods.

Protein	Range of concentration and number of lumbar liquores used		Comparison of techniques	Regression equation (y on x; mg/l)	Correlation coefficient
	(mg/l)	(n)			r
Prealbumin	20–70	(20)	Radial immunodiffusion vs. Behring laser nephelometry	y = 1.09 x – 0.44	0.982
Prealbumin	14–72	(115)	Behring laser nephelometry vs. turbidimetric end point analysis	y = 1.04 x – 0.16	0.939
Albumin	100–1000	(71)	Hyland laser nephelometry vs. Behring laser nephelometry	y = 1.02 x – 4.76	0.884
Albumin	70–1000	(72)	Behring laser nephelometry vs. turbidimetric end point analysis	y = 0.98 x – 3.75	0.906
IgG	5–95	(52)	Hyland laser nephelometry vs. Behring laser nephelometry	y = 1.04 x + 0.23	0.922
IgG	14–170	(39)	Hyland laser nephelometry vs. Beckman immunochemistry analyser (cf. 4)	y = 1.27 x – 3.24	0.962
IgG	15–90	(65)	Hyland laser nephelometry vs. turbidimetric end point analysis 1.5 h (cf. l.c. (11))	y = 1.16 x – 1.06	0.963
IgG	11–100	(79)	Hyland laser nephelometry vs. turbidimetric end point analysis 20 min	y = 1.03 x – 5.99	0.990
IgA	0.06–1.4+	(47)	Hyland laser nephelometry vs. Behring laser nephelometry	y = 0.82 x + 3.74+	0.960
IgM	3.5–16.1	(39)	Hyland laser nephelometry vs. Behring laser nephelometry	y = 1.01 x – 0.01	0.980

+ IU/ml

(fig. 8) with polyethylene glycol in the assay (concentrations used between 19–35 g/l). For comparison, IgG concentrations in CSF determined by Hyland and Behring laser nephelometry (the latter with polyethylene glycol) also correlated well (tab. 9). Both correlations exhibited a y-intercept below the detection limit of the tests (tabs. 5,9). Turbidimetric end point analysis without polyethylene glycol and Beckman immunochemistry analyser yielded somewhat higher values of IgG concentrations in CSF samples. This could be caused by different IgG standards, or blank values that are too low (cf. l.c. (14) and unpublished results).

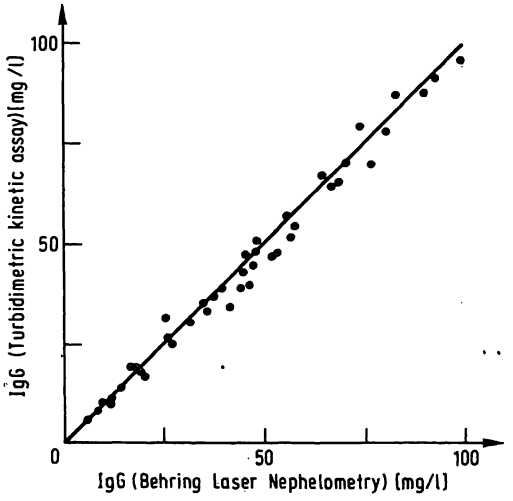

Fig. 8. IgG concentrations in CSF as determined by turbidimetric kinetic assay (Tina quant, see tab. 4) and Behring laser nephelometry (see tab. 2) (n = 40).
Slope: 0.97, intercept: 0.33 mg/l, correlation coefficient: 0.983.

Difficulties appear in the correlation of *IgA* concentrations determined by three different techniques, using different standards (cf. l. c. (14)). The concordance obtained between Hyland laser nephelometry and Behring laser nephelometry was unsatisfactory, even when values were calculated in IU (international units according to WHO, cf. l.c. (13)) for both standards: the slope of the linear regression curve became < 0.9 indicating higher IgA values for Hyland laser nephelometry (tab. 9). According to the data of both manufacturers Hyland IgA standards appear to be ~25% higher than Behring standards related to the W.H.O. standard. After correcting the slope by this factor, it becomes ~1, indicating a good correlation for IgA values in CSF for both techniques.

The concordance obtained between the turbidimetric kinetic assay and Behring laser nephelometry was good (fig. 9). After adding higher concentrations of polyethylene glycol (≥ 13.3 g/l) to the nephelometric end point assay (Behring laser, see tab. 2), higher IgA concentrations were obtained in CSF samples than in comparison to Hyland laser nephelometry (see tab. 1). It was also the case with Behring laser nephelometry without polyethylene glycol (slope ~ 1.16, r = 0.957, n = 57).

All these difficulties were mainly caused by a lower standard curve which had a lower slope in the presence of polyethylene glycol (after 2 h incubation time) thus yielding higher IgA concentration in CSF samples. The results point to the presence of different IgA species in CSF and control serum.

Good agreement was obtained between turbidimetric end point assay and Hyland laser nephelometry for *IgM*

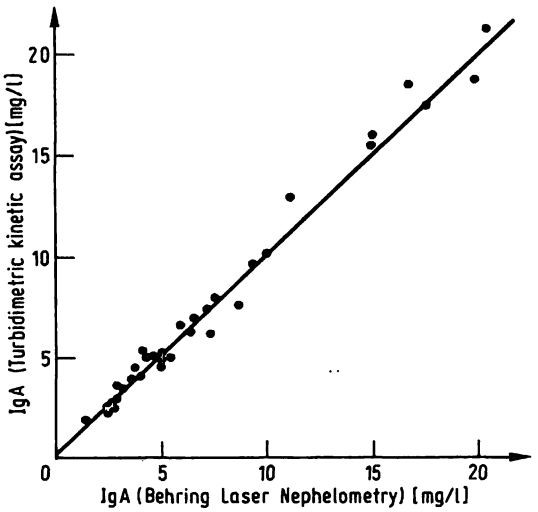

Fig. 9. IgA concentrations in CSF as determined by turbidimetric kinetic assay (Tina quant, see tab. 4) and Behring laser nephelometry (see tab. 2) (n = 37).
Slope: 0.98, intercept: 0.23 mg/l, correlation coefficient: 0.978.

(fig. 10) as well as between Behring laser nephelometry (without polyethylene glycol) and Hyland laser nephelometry (tab. 9). However, after adding higher concentrations of polyethylene glycol (≥ 13,3 g/l) to the Behring laser assay nearly every CSF sample yielded measurable IgM values indicating unspecific precipitation of proteins.

In summary, the presence of polyethylene glycol considerably accelerates turbidimetric assays for the five proteins in CSF, without disturbing their correlation with the reference procedures. However, the addition of high polyethylene glycol concentrations to nephelometric assays needing sample volumes > 50 µl

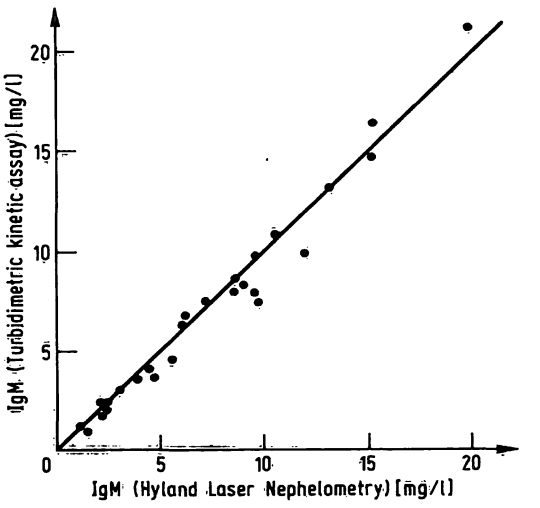

Fig. 10. IgM concentrations in CSF as determined by turbidimetric end point assay (0.5–25 min, antiserum mixture (Tina quant) concentrated by a factor of 4, 34 g/l polyethylene glycol, conditions see tab. 4) and Hyland laser nephelometry (see tab. 1) (n = 31).
Slope: 1.01, intercept: −0.26 mg/l, correlation coefficient: 0.960.

for the detection of low amounts of protein, led to non-correlative values.

c) *Calibration curves prepared with* samples of *lumbar CSF* containing high amounts of prealbumin, IgG, IgA and IgM, and with albumin exhibited a good linearity for the turbidimetric and nephelometric techniques (not shown here). However, this was also the case in the presence of high concentrations of polyethylene glycol in the assay even when no correlation of the values was obtained with other techniques.

Practicability of turbidimetric and nephelometric techniques

Generally, turbidimetric kinetic analysis requires less time to determine a few samples (≤ 10) than the nephelometric end point techniques (by a factor of 2 to 4, see tab. 10). This is because the incubation period is shorter, and the turbidimetric kinetic assay requires no calibration curve for each series (see above). The difference in time between both techniques diminishes with turbidimetric end point assays. It also becomes smaller with a greater number of samples (table 10). The shortest time per sample for small and large numbers of analysis is required with the Beckman immunochemistry analyser (tab. 10). However, this technique is only suitable for CSF with rather high concentrations of IgG; for IgA and IgM it is too insensitive (cf. l.c. (4)). End point measurements are necessary for IgA and IgM with this technique, which consumes as much time as the techniques described here.

The approximate costs of the reagents for one specimen and 10 or 30 specimens were lowest for turbidimetric kinetic assays for albumin, IgG and IgA and turbidimetric end point assay for IgM compared to both the nephelometric assays and Beckman immunochemistry analyser with respect to IgG (tab. 10). They can be further lowered with turbidimetric assays by a factor of 2 to 20 by omitting the daily calibration curve (tab. 10). Under these conditions the turbidimetric kinetic assay for a few samples of prealbumin also becomes cheaper than the nephelometric one (tab. 10). The costs of reagents for turbidimetric end point assays for prealbumin, albumin and IgG lay in the range of those of turbidimetric kinetic assays. The advantage of turbidimetric micro-assays disappears when semi-micro-assays of ~1 ml total volume are used.

Comments

The manual turbidimetric kinetic and end point assays described for prealbumin, albumin, IgG, IgA and IgM consume less time, cost less for reagents and usually require less sample volume than the two nephelometric end point assays.

Tab. 10. Comparison of technician time and approximate costs of nephelometric and turbidimetric techniques for the determination of five proteins in CSF.

Protein	Technique	Requirements per sample Technician time (min) for n[a]) = 1	n = 10	n = 30	Cost (DM) of reagents and supplies[b] n = 1	n = 10	n = 30
Prealbumin	Behring laser nephelometry	45 (105)[c]	6 (12)[c]	3 (5)[c]	22.80	3.65	2.25
Prealbumin	Turbidimetric kinetic analysis	15 (55)[d]	5 (9)[d]	5 (6)[d]	5.65 (44.85)[e]	3.15 (7.05)[e]	2.95 (4.25)[e]
Albumin	Hyland laser nephelometry	45 (105)[c]	6 (12)[c]	3 (5)[c]	50.45	7.60	4.45
Albumin	Behring laser nephelometry	45 (105)[c]	6 (12)[c]	3 (5)[c]	20.10	3.20	2.00
Albumin	Turbidimetric kinetic analysis	10 (50)[d]	3 (7)[d]	3 (4,5)[d]	2.10 (16.10)[e]	1.15 (2.55)[e]	1.10 (1.60)[e]
IgG	Hyland laser nephelometry	45 (105)[c]	6 (12)[c]	3 (5)[c]	37.85	5.70	3.35
IgG	Behring laser nephelometry	45 (105)[c]	6 (12)[c]	3 (5)[c]	16.50	2.75	1.70
IgG	Beckman immunochemistry analyser	3.5	1.5	1.5	3.90	3.90	3.90
IgG	Turbidimetric kinetic assay B	10 (50)[d]	3 (7)[d]	3 (4,5)[d]	1.45 (11.95)[e]	0.90 (1.85)[e]	0.75 (1.15)[e]
IgA	Hyland laser nephelometry	45 (165)[c]	7 (19)[c]	4 (8)[c]	36.05	5.45	3.15
IgA	Behring laser nephelometry	45 (165)[c]	6 (18)[c]	3 (7)[c]	22.00	3.60	2.25
IgA	Turbidimetric kinetic assay B	15 (55)[d]	5 (9)[d]	5 (6)[d]	2.45 (20.45)[e]	1.40 (3.20)[e]	1.25 (1.90)[e]
IgM	Hyland laser nephelometry	45 (165)[c]	7 (19)[c]	4 (8)[c]	34.25	5.20	3.05
IgM	Turbidimetric end point assay 0.5–25 min, 34 g/l polyethylene glycol	30 (85)[d]	6 (12)[d]	6 (8)[d]	3.05 (25.55)[e]	1.70 (3.95)[e]	1.60 (2.35)[e]

[a]) n = number of specimens per series, [b]) cost of antiserum, standards, control sera, reagents and pertinent supplies per sample, [c]) time including incubation period, [d]) time including standard curve, [e]) costs without standard curve.

The turbidimetric kinetic assay in series of 6 samples can be compared to the Beckman immunochemistry analyser which needs still less time, but it is less sensitive and more costintensive. These turbidimetric techniques can be well compared to other manual (15, 16) and automated (17) immunological procedures for the determination of proteins in CSF.

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